

# The Canadian Medical Association Journal

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VOL. II.

APRIL, 1912

No. 4

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## THE ESTABLISHMENT OF TREPONEMA PALLIDUM AS THE CAUSATIVE AGENT OF SYPHILIS, AND THE CULTURAL DIFFERENTIATION BETWEEN THIS ORGANISM AND CERTAIN MORPHOLOGICALLY ALLIED SPIROCHÆTÆ

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**C**LINICIANS have long recognized the infectious nature of syphilis, but it is only since the inauguration of scientific microbiology by Pasteur, Koch, and their pupils, that the cause of the disease has been more zealously studied. A definite ætiological relation was soon established, step by step, between certain parasitic microorganisms and the morbid processes constantly associated with them. According to Koch, the following conditions must prevail in order that this relation exist. The organism must be found constantly in sufficiently large numbers; it must not be found in other diseases; it must be capable of producing the same pathological changes when introduced into a suitable host in pure culture as it does in the disease of which it is supposed to be the cause. Since the laying down of the above requirements by Koch, other important phenomena, such as immunity, anaphylaxis, and allergy, have been proven to become essential factors in determining the ætiological relation between an organism and a given disease. Indeed, only through definite, immunity phenomena were certain pathogenic organisms first recognized as ætiological factors of a number of infectious diseases.

While the causative agents, whether visible or invisible, of

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Read before the Ottawa Medical Society, February 9th, 1912.

some of the most important diseases have been discovered and identified, there is still a large number of diseases the causative agents of which are still unknown. There are, however, certain groups of diseases in which the causative microorganisms are doubtless known, since they are constantly found in the same disease, but their ætiological relation cannot be conclusively determined, chiefly because of the lack of adequate methods of isolating the suspected organisms. In other words, a pure culture of the organism is required. This was the situation with *Treponema pallidum* until I succeeded in producing pathological changes indistinguishable from human syphilitic processes by means of pure cultures of this organism. The search for the causative organism of syphilis had been made by a great many investigators. It was not, however, until 1905, when Schaudinn and Erich Hoffmann discovered a distinct type of spirochæta now known as *spirochæta pallida*, or *Treponema pallidum*, that an organism present in sufficient number in syphilis, and never in any other disease, became known to us. This remarkable discovery of Schaudinn and Hoffmann was rapidly confirmed throughout the world, and the presence of this organism in any lesion is accepted as the definite proof of syphilitic nature. In this connexion we must not forget the great service rendered by Metchnikoff and Roux, who had previously shown that the virus of syphilis is transmissible to certain apes and that it does not pass through the pores of a Chamberland filter. The latter fact is important to the investigator, since it shows the virus to be large enough to be seen under the microscope. In spite of the slight refractory property possessed by this organism, Schaudinn was able to see it without staining. He subsequently found a stain in Giemsa's solution, which brought a faint colour (hence called *pallida*) in fixed specimens. Metchnikoff and Roux next demonstrated this organism in the lesions experimentally produced in apes. Further progress was then made by E. Hoffmann, Buschke, Fischer, Tomaszewski, Bertarelli, Volpino, Levaditi, Uhlenhut, Mühlens, Neisser, Truffi, Mulzer, Flexner, Nichols, and many others, who not only succeeded in demonstrating the enormous number of *Treponema pallidum* in acquired as well as congenital syphilitic lesions and organs, but also in the tissues (chancre, orchitis, keratitis) of certain lower animals, such as rabbits, that have been inoculated with the human material rich in *pallida*. The result of these investigations was that the *pallida* was regarded as the cause of syphilis. The final proof of this, however, depended upon the ability to grow a pure culture of *Treponema pallidum*.

The pure cultivation of *Treponema pallidum* opens up many important problems in syphilis. By means of it, we can study the biological characters of the organism more closely and definitely, and investigate the phenomena of immunity and anaphylaxis which are most likely to develop during the chronic course of the disease in man. Since the discovery of the organism by Schaudinn and Hoffmann in 1905, numerous bacteriologists have attempted to cultivate it in artificial media, but no success was reported until 1909, when Scherschewsky announced that *Treponema pallidum* could be grown in a gelatinized horse-serum together with many other associating bacteria. This has since been confirmed by other investigators, who, like Scherschewsky, have all failed to isolate it. During 1909 and 1910 Mühlens reported that he obtained a pure culture from a mixed growth in Scherschewsky's horse-serum, having employed a mixture of horse-serum and agar for purification. Mühlens carefully stated that his spirochæta was morphologically indistinguishable from the pallida and produced a strong putrefactive odour in the culture. The appearance of growth was similar to that of the culture of spirochæta dentium. Mühlens' culture was absolutely non-pathogenic for monkeys, rabbits, and certain other laboratory animals. Continuing the work of Mühlens, W. H. Hoffmann in 1911 claimed to have cultivated five more strains of the same organism from human lesions by the same process. This investigator does not give any detail of the morphology or biology of his cultures except to state that they were identical in every respect with those isolated by Mühlens. He stated, however, that his strains grow even in plain agar without horse-serum. The cultures of Mühlens and W. H. Hoffmann are anærobic. While Mühlens could not produce any lesion with his culture, W. H. Hoffmann in his recent paper claims to have produced an orchitis in the rabbit with his cultures, a condition which he could not accomplish at the time of the publication of his first report. Whether this organism with the morphology of spirochæta pallida, easily cultivatable in a mixture of horse-serum and agar and capable of producing a fetid odour in its growth, is the real pallida or a spirochæta resembling the pallida merely in morphology, will be shown later when we discuss the mouth spirochætæ cultivated by me. In the meanwhile, Galasesco and Brückner in Roumania, and Sowade in Germany, succeeded in producing characteristic lesions in the rabbit by means of impure cultures of the pallida grown in Scherschewsky's horse-serum.

Independently of these investigators and employing entirely

different methods, I succeeded in 1910 and 1911 in obtaining several pure cultures of *Treponema pallidum* and one of *Treponema pertenue* (yaws) from the orchitis produced in rabbits by transmitting the spirochætæ from human cases. The reason for choosing the orchitis material of the rabbit was to exclude the possibility of cultivating another spirochæta resembling the pallida and confusing it with the latter. The orchitis material contains almost pure pallida and no other similar saprobiotic forms, such as the dentium, which must be taken into consideration when isolating the pallida from a chancre or condyloma in human cases. I will not describe here the exact technique for the pure cultivation of the pallida; I refer the reader to my papers on this subject. With six different pure strains of the pallida, I was able to reproduce typical orchitis in the rabbit. After familiarizing myself with the cultivated pallida, I proceeded to isolate the organism directly from human chancres, condylomata, and skin papules, the mouth lesions being avoided on account of the presence of the dentium which, as will be shown, is difficult to differentiate from the pallida in morphology. Thus far seven different strains of the pallida have been isolated and are kept growing in a pure state. The morphology and biology of these agree with those of the strains previously isolated from rabbit orchitis, and are found to produce characteristic lesions of the skin in certain monkeys. The Wassermann reaction developed also after successful inoculation.

The general characteristics of the cultivated pallida are: (1) strict anærobiosis; (2) requirement of fresh sterile animal or human tissue and serum (or ascitic fluid) in the culture medium for growth; (3) almost transparent, diffuse growth, seldom forming a discrete colony; (4) no coagulation of serum or tissue constituents; (5) no production of any putrefactive odour in growth; (6) optimum growth in a weak, alkaline reaction and at a temperature of about 37° C.; (7) beginning of growth after several days and its continuation for several weeks, majority of the organisms retaining their motility; (8) the presence of a long, straight, or finely curved, delicate flagella-like projection at one or both ends; (9) longitudinal division.

For comparative purposes I have also cultivated for the first time two different species of common mouth spirochætæ; namely, one heavier, and one delicate type, designated by me *Treponema macrodentium* and *microdentium*, respectively. The *microdentium* was known as *spirochæta dentium* (Koch) before cultivation, and the *macrodentium* bore no particular name, although it was called by

some authors medium form. Before their isolation in pure cultures, it was not known whether both forms were varying sizes of the same organism or two different species.

The most important of the two dentiums is the microdentium, because this organism is almost impossible to differentiate morphologically from the pallidum, and hence it might be confused with the latter. Only those experienced with both cultures may detect certain differences in morphology. On the other hand, nothing is easier than to pick out the microdentium from the pallidum when both are compared in cultural characteristics. The following points are sufficient to distinguish the two: (1) the microdentium grows more easily and rapidly and forms more discrete and denser colonies than the pallidum; (2) it is less strictly anærobic; (3) it produces a putrefactive odour; (4) it grows without the addition of a fresh sterile tissue to the media; (5) in an old culture the tissue, if added to the media, assumes a dark brown to blackish colour; (6) in a good growing fluid culture the serum water may be loosely coagulated after several weeks. The macrodentium is more easily differentiated from the pallidum by its irregular curves, peculiar vibrating motility, and its general appearance, although in growth it resembles the pallidum closely, especially as it produces no odour.

In view of these facts, one wonders how the so-called pallidum strains of Mühlens and W. H. Hoffmann can be distinguished from the microdentium with which they agree in every respect. Furthermore, their pallidum strains are entirely different from the author's in most fundamental, biological characteristics. This question is also raised by Erich Hoffmann in his latest work, in which he further points out that in any syphilitic organ containing enormous numbers of the pallidum there is no odour, hence the cultures of Mühlens and W. H. Hoffmann must contain, besides the pallida, certain spirochæta capable of producing the characteristic putrefactive odour. But, as their culture medium contains no fresh tissue and their strains continued to grow through numerous transfers, whereas no pallida can grow under the same conditions, it is doubtful whether their cultures contained any pallida. It is more probable that they have been dealing with the microdentium, or at least a spirochæta indistinguishable from it in cultural properties.

In order to use the pure culture of *Treponema pallidum* for advancing our knowledge in the study of syphilis, we naturally turn to the field of immunity and anaphylaxis. I will not discuss the question of immunity here, but I will limit myself to the phenomenon of local anaphylaxis, or allergy, as termed by von Pirquet,

who first discovered similar reaction in tuberculosis. Syphilis, like tuberculosis or certain other chronic infectious diseases, confers upon the patient a hypersensitive condition of the tissues, that is, a skin allergy is established after a certain period in the course of the disease. This condition is easily detected by introducing a small quantity of the devitalized pure culture of *Treponema pallidum* (luetin) into the epidermis. If the allergy is already present, the inoculation of the luetin is followed by a marked inflammation at and about the site of injection, comparable to von Pirquet's tuberculin test for tuberculosis. While referring the reader to the detailed report, I may state briefly that I found the allergic state of skin in syphilis to be present in almost every case of the tertiary stage and in a large percentage of latent and late hereditary syphilis. It was almost always absent in primary and very early secondary syphilis, in which stages this condition may be brought out by means of an energetic anti-syphilitic treatment. It is especially striking to observe the appearance of allergy in the cases of secondary syphilis after the injection of salvarsan. This is apparently due to the destruction of a large number of *Treponema pallidum* in the human body by this powerful spirochætocide and subsequent absorption of the pallidum substances by the entire system, including the skin. That this is true is shown by the fact that even rabbits can be made allergic through repeated injections of the dead or living pallida, as was demonstrated by me. Normal individuals or those suffering from non-syphilitic diseases never give the reaction.

The diagnostic value of the luetin test has its own sphere, as have other diagnostic methods. It does not give any reaction until the allergic state of the skin develops, but after its appearance it seems to persist until the probable eradication of the disease from the system, and remains uninfluenced by the ineffective treatment. In this respect the test appears to have a prognostic value, which neither the negative clinical symptoms nor serological examinations possess. During the late stage of syphilis, when visceral organs are affected, and the clinical and serological examinations yield no decisive diagnosis, the luetin test assumes an important position in determining the nature of the affection. In other words, the early stages of syphilis can best be diagnosed by the demonstration of the pallidum, by the clinical manifestations, and by the serological examinations; the late stages by resorting to the luetin test. I have met with a number of cases of hereditary syphilis in which, despite the strong serum reaction and extensive clinical manifestations, no luetin reaction was obtained. I consider these cases of a bad prognostic

character, as this only indicates that the disease is too severe to enable the patients to respond to the infecting agent by the development of allergy. Thus, it will be seen that the intensity of the infection is, with certain exceptions, in direct ratio to the severity of the clinical and serological manifestations; while the luetin reaction indicates either that the infection is well borne by the patients or that it is under a better control, due to the treatment. Thus it is natural to find cases with a negative Wassermann reaction, and positive luetin test, although both reactions are found coexisting in many instances.

The above estimate of the luetin test is derived from my observations of several hundred cases, but its ultimate significance and proper utilization in practice will be demonstrated only through careful observations made by clinicians in different fields of medicine in the future.

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